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14. ABSTRACT

The long-term goal of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin hey represent an in vivo test bed to examine both tumor biology and the application of therapeutic agents. In the second year of funding we have made a thorough study of the profiles of metastatic spread of human prostatic epithelium from the sub-renal and orthotopic graft sites and have found that the orthotopic site shows spread patterns and mechanisms which closely profile the human disease and apparently result from similar migration routes. We have developed a novel method of intraductal orthotopic grafting which enhances the efficiency of tumorigenesis and metastasis at the orthotopic site. We have further explored the use of lower dose Myc expressing constructs and have investigated the combination of lower levels of Myc with other genes commonly changed in prostate cancer to make more clinically relevant models. Tet- regulated Myc constructs have been used to develop tumors with suitable profiles in mice.

15. SUBJECT TERMS
tissue, recombination, prostate cancer, in vivo, metastasis, c-Myc, oncogene, model

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Introduction

The **long-term goal** of the work proposed here is to generate, characterize and interrogate human epithelial cell-based in vivo models of prostatic carcinogenesis. These models will allow an examination of processes involved in carcinogenesis, tumor growth and metastasis. Since the tumors are themselves of human origin they represent an in vivo testbed to examine both tumor biology and the application of therapeutic agents.

The first two aims will generate data on cancer development, androgen dependence, gene expression and patterns of metastatic spread prostate samples overexpressing c-Myc. The third specific aim will use this information to target specific genes and normalize their expression in the Myc tumor. If a positive phenotype results the gene product may be a potential therapeutic target.

We are using models in which human prostatic epithelial cells (huPrE) are grown in tissue recombinants with rat urogenital sinus mesenchyme (rUGM) and grafted back into the in vivo environment of an intact male athymic rat host. Manipulations of the huPrE allow us to examine the effects of retroviral transfection with c-Myc within the huPrE. Our original C7-Myc model forms aggressive tumors which move rapidly from a benign to metastatic phenotype. Hence, as originally proposed, we are making less powerful and externally regulatable constructs that will allow us to follow the progressive events in cancer initiation and progression. In combination with this approach we are able to modify specific oncogenes and tumor suppressor genes to examine their effects in combination with those of c-Myc. It is highly desirable to study prostate cancer formation/progression in human prostatic epithelial cells, in an in-vivo setting, in order to minimize cell culture artifacts and more fully understand prostate cancer in vivo.

Work Ongoing and Completed

The first specific aim of this proposal was to characterize the C7-Myc model which we had developed. As noted in the first annual report and in the published paper on the subject (Williams et al), which described some parts of the work proposed in specific aim 1, the C7-Myc tumor turned out to be an extremely aggressive and fast growing model and is therefore of limited use for the sorts of studies which are envisioned for this approach to modeling human disease. We have therefore explored (as proposed in specific aim 2) a number of options to develop models which are more relevant to human disease. With this in mind the tetracycline-inducible system has been transduced into prostate epithelial cells to examine the effects of regulatable lower doses of c-Myc on prostate cancer progression. Several

independent transductions have been performed and the cell lines collected and frozen after exposure to the oncogene in culture. The tissue recombinant grafts of urogenital sinus mesenchyme plus epithelial cells containing tetracycline inducible Myc were grafted to SCID mouse hosts and allowed to establish prior to activation of the transgene. Several animals have now been sacrificed and grafts harvested for histology.

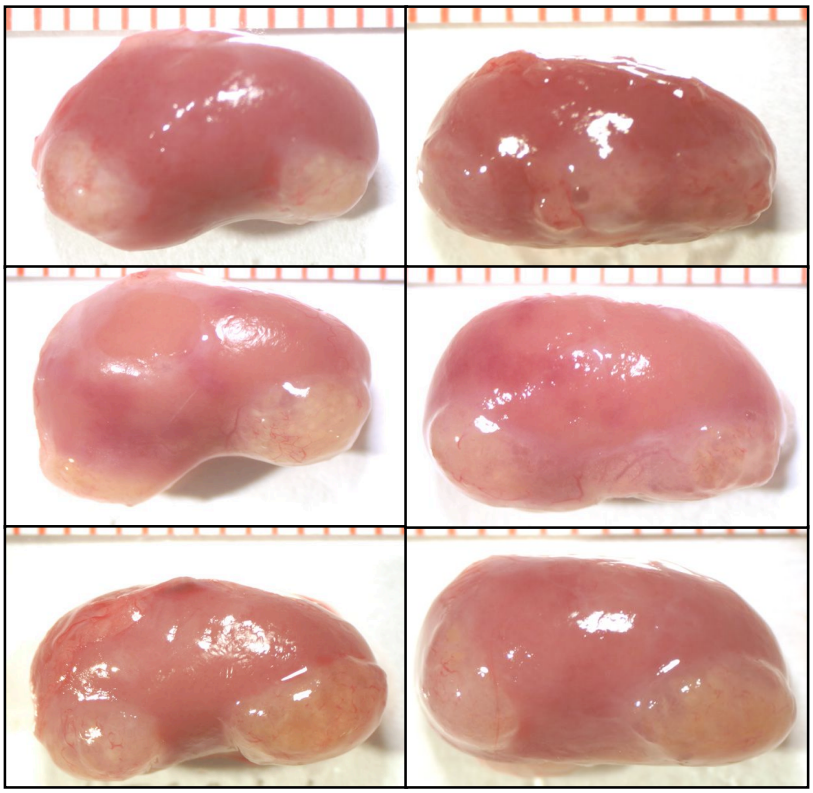

Figure 1. Expression of c-Myc in AR positive and negative 957e/hTert human prostatic epithelial cells (gift from Dr. John Isaacs, Johns Hopkins Medical School).

Left hand panel
AR positive cells transduced with TET inducible virus. Cells matured and organized for 4 months prior to switching on of transgene. Tetracycline administrated for 3 months.

Right hand panel
AR negative cells transduced with TET inducible virus. Cells matured and organized for 4 months prior to switching on of transgene. Tetracycline administrated for 3 months

Gross pictures of the grafts (figure 1) indicate that the tumor are considerably smaller and less aggressive than the C7-Myc tumor. The transduced cells represent both androgen receptor positive and androgen receptor negative tumors depending upon the presence or absence of the AR in the epithelial cells used to make the grafts. Since the stromal environment contains AR this does not necessarily reflect the androgen responsiveness of the tumors, a feature which is now under investigation as proposed in specific aim 1. Histologically, these tumors exhibit widespread areas of cellular piling and some cribriforming with nuclear atypia consistent with a premalignant PIN histology. Smaller regions of associated invasive cancer were also seen. These data suggest that it is possible to model the early stages of prostate cancer in these immortalized cells with appropriate (reduced) doses of c-Myc. These data

also indicate that this model will prove suitable for use as an experimental platform for the microarray studies proposed in specific aim 3. Work is ongoing to generate RNA for the microarrays.

In addition to the use of tetracycline-regulated c-met expression we have developed a lentiviral vector using the viral LTR as a promoter to constitutively express c-may at a low level. Data from transgenic mouse studies suggest that the use of a range of doses of c-Myc should give a range of phenotypes and can be used in conjunction with modification of other oncogenes and tumor suppressor genes relevant to human prostate cancer to mimic different disease states, from disease initiation to more aggressive cancer. These studies have put us in a good position to rapidly complete the androgen ablation studies proposed in specific aim 1b.

Consistent with this approach and in accordance with the concepts outlined in specific aim 3 we have examined a series of human prostatic epithelial cell lines in tissue recombinants with rat urogenital sinus mesenchyme to assess candidates genes to combine with c-Myc. Consistent with our previous experience primary cultures of human prostatic epithelium form prostatic structure when recombined with rUGM. However some specific genetic changes result in cells which undergo pre-malignant changes. An example of this is shown in figure 2.

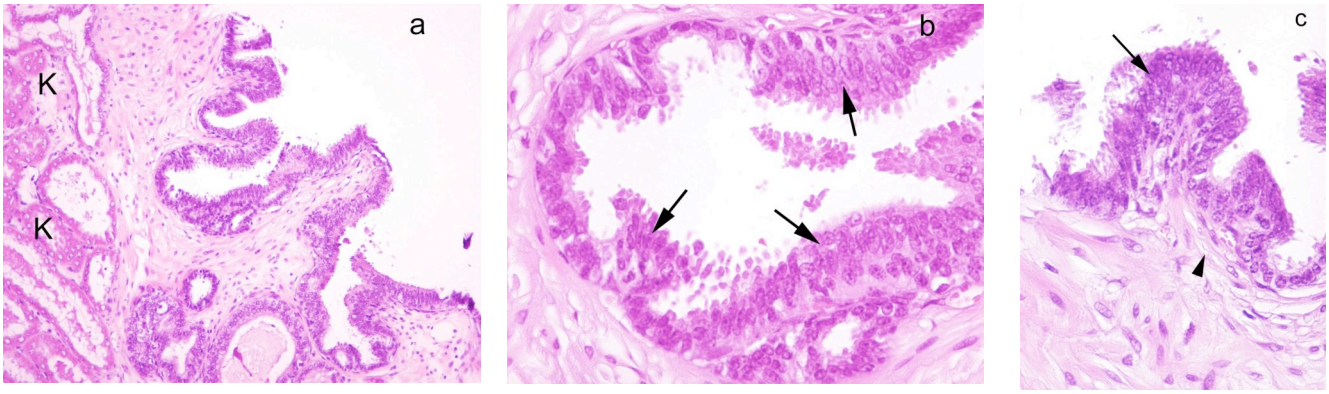

Figure 2. Tissue recombinant of rUGM and primary human prostatic epithelium expressing hTERT and shRNA targeting p16. At three months post grafting to a SCID mouse host many areas of tufting characteristic of PIN are seen (arrows). Host kidney (K)

A number of cell lines have been examined. These include PrE5 which was immortalized by the expression of SV40 large T antigen. Tissue recombinants using these cells demonstrate reasonably normal histology (figure 3). However this organization can be disrupted with by either suppression of PTEN or overexpression of a constitutively active form of the downstream molecule Akt. The validation

of these cell lines and the development of the viral constructs used to modify gene expression provides and validates the tools that will be needed in the second part of specific aim 3.

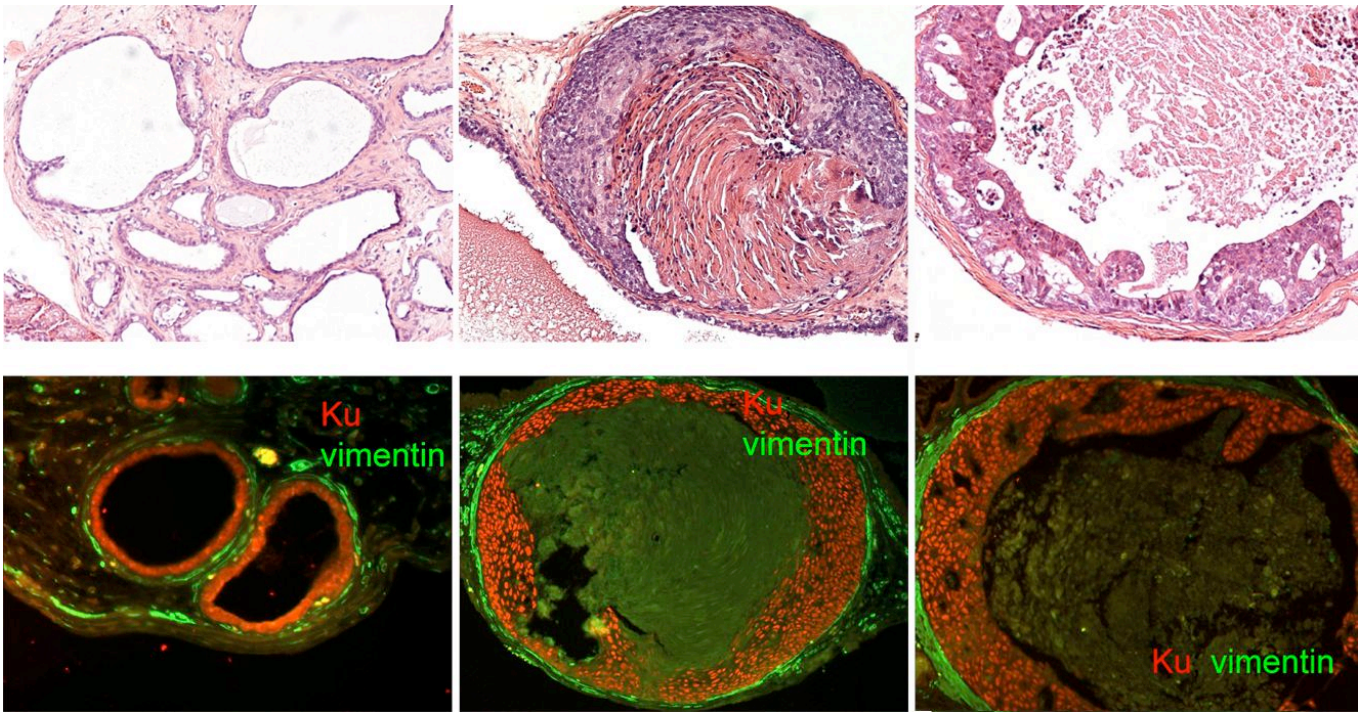

Figure 3. Recombinants of PrE5 human prostatic epithelial cells (express SV40T antigen) with rat UGM gives rise to glandular structures (left panels). The human origin of the epithelium is confirmed by Ku2 staining visualized in red. Suppression of PTEN (center panels) or overexpression of myristylated Akt (right panels) affects the histology of these human prostatic epithelial cells resulting in PIN-like lesions. Note increased vimentin expression adjacent to the modified epithelial cells, consistent with phenotypes seen in human prostate cancer

Specific aim 1a focusses on characterizing metastatic spread in Myc expressing models of prostate cancer. To best assess this phenomenon we have developed a new orthotopic grafting method using intraductal grafting of human prostatic epithelial cells to produce an in vivo model which in many ways closely resembles human prostate cancer. This model metastasizes in a pattern very similar to that seen in human prostate cancer patients including metastasis to bone. The specific advantages of intraductal grafting seem to be that tumors initially form within the prostatic ducts. This allows them to develop in a manner which mimics human prostate tumors growing in patients. The resulting metastatic spread pattern also follows a pattern similar to that seen in human patients with the tumor cells migrating along the spinal column and invading the spine and major bones as well as the liver, lungs and other organs (illustrated in figures 4 and 5). This is important because metastatic spread to the bone is an important biological component of human prostate cancer which has not been easy to model in the in vivo systems

used historically. In contrast, metastasis from the renal capsule site is generally restricted to the renal lymph nodes except in extremely aggressive tumors such as the C7-Myc model where it follows the lymphatic and vascular drainage of the kidney resulting in metastatic profiles which do not resemble that seen in prostate cancer patients.

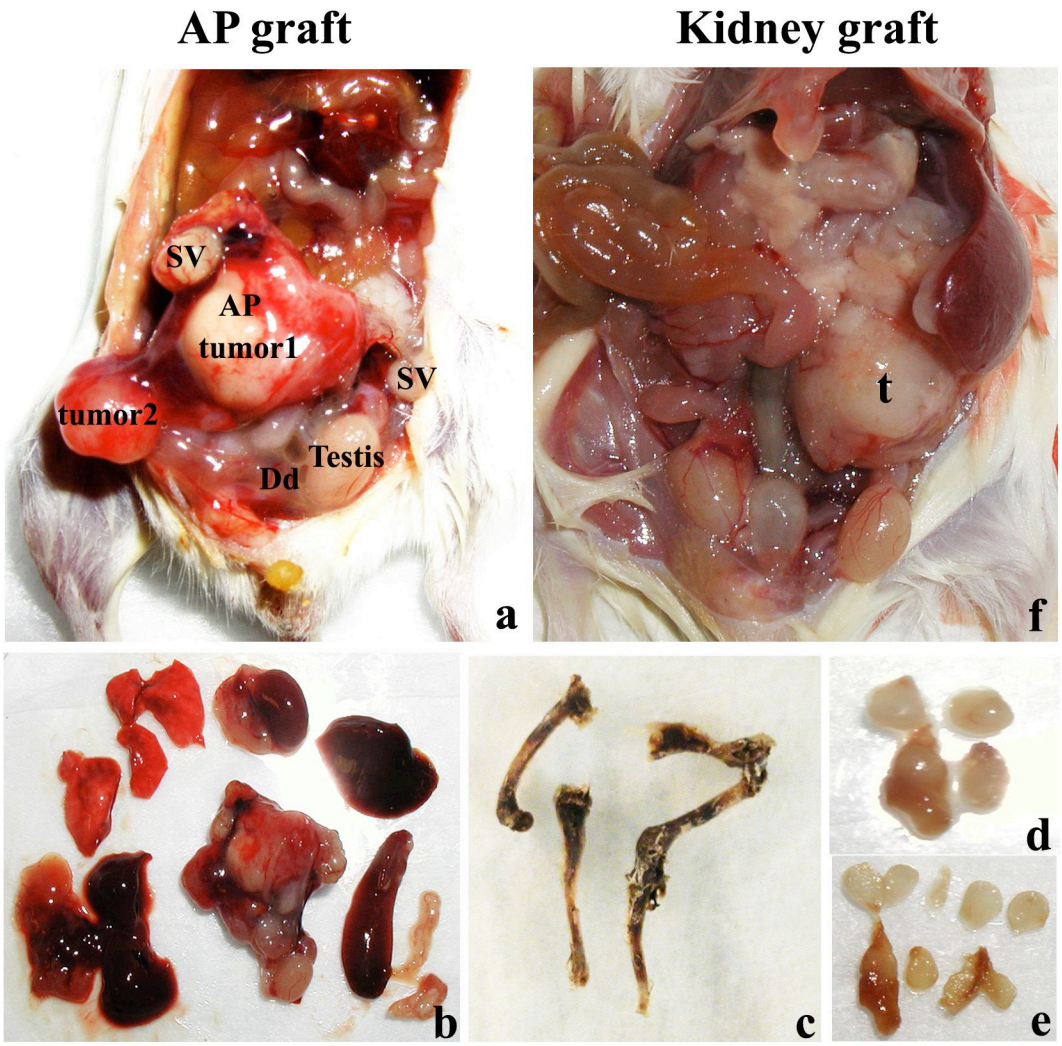

Figure 4. Gross appearance of intraductal-anterior prostate xenografting compared to a sub-renal capsule grafts. (a-e), PC-3-EGFP cells (200K) in collagen gel were introduced to the intraductal-AP grafting site. 7 weeks post implantation metastasis to many organ sites had occurred. (a) Gross appearance prior to dissection. (b) gross appearance of multiple visceral organs showing metastatic lesions in liver and lungs but no gross lesions in kidney or spleen. (c) metastases to long bones (femur and tibia), (d and e), enlarged and hardened pelvic lymph nodes. (f) The same cells grafted to the sub-renal capsule of a SCID mouse for the same time period resulted in a large local tumor but no evidence of metastatic spread.

Histologically the invading tumor cells were identifiable as both locally invasive and metastatic lesions. As shown in figure 5

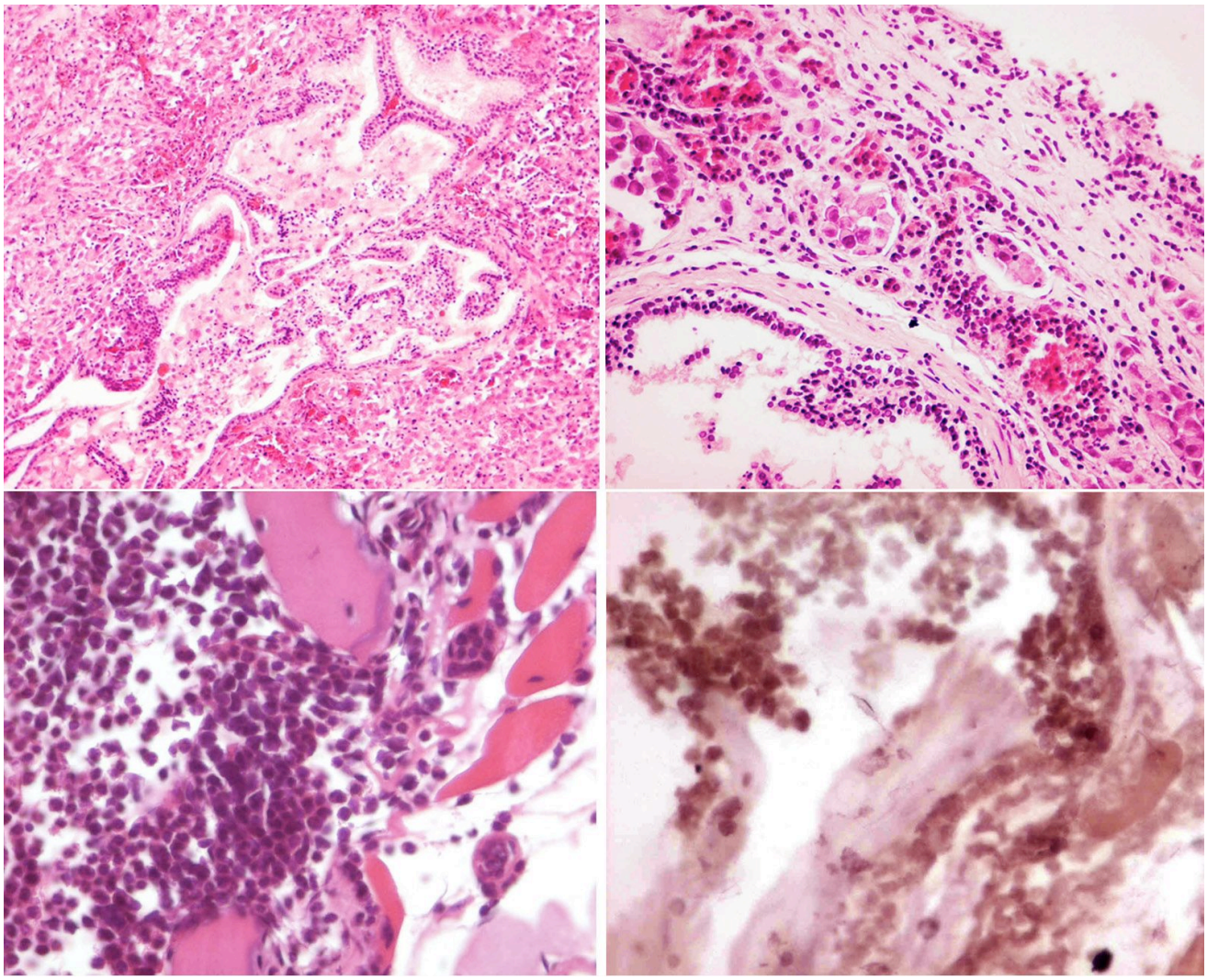

Figure 5. Appearance of local invasion (top panels) and bone metastases (bottom panels) from intraductal grafts to the anterior prostate. Tumor cells can be seen both within the mouse prostatic ducts (top panels) and invading locally to surround and enter small local blood vessels (detail in right panel). At the bone site (bottom panels) metastatic cells can be seen, their human identity confirmed using an antibody against human mitochondria (bottom right panel).

Technical Modifications

We have modified the orthotopic site graft method to use intraductal grafting which has proven to give a more reliable pattern of metastatic spread, more closely resembling that seen in human prostate cancer patients. We have also included the use of a number of human prostatic epithelial cell types which were not available at the time this proposal was written. These allow more consistent data to be generated than the proposed use of primary epithelial cultures.

Personnel Changes

Dr. Karin Williams has moved to take up a faculty position at the University of Rochester (New York) her post-doctoral role on this project has been taken by Dr. Ming Jiang.

Key Research Accomplishments

- Development of a new model of prostate cancer metastasis based upon orthotopic intraductal xenografting.
- Development and characterization of tet-regulated c-Myc models using human prostatic epithelium.
- Characterization of viral vectors to suppress PTEN expression and to activate Akt signaling in tissue recombinants using human prostatic epithelium and rUGM.

Reportable Outcomes.

None

Conclusions.

This work is proceeding on the predicted timeline. A number of changes to the specific details of the original statement of work are noted. These reflect technical methodological improvements that enhance the overall quality of the proposal. The second year of work has been devoted to generating and characterizing model systems which will allow us to proceed rapidly to complete the work.